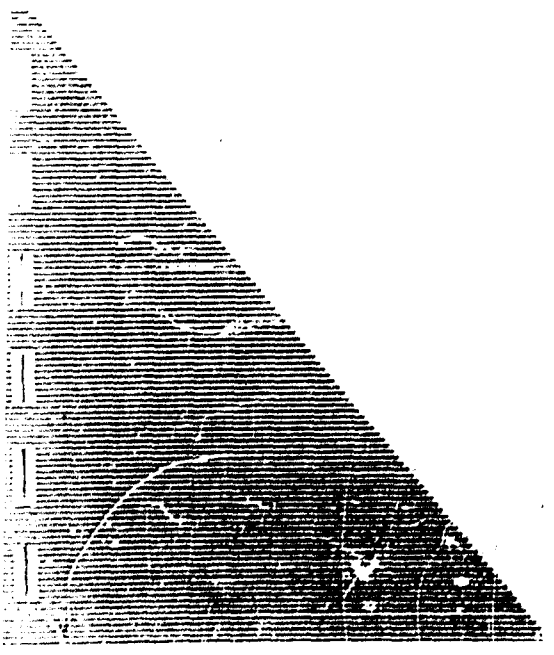

NOTE: Effective June 1, 1963, the name of Armour Research Foundation of Illinois Institute of Technology will change to IIT RESEARCH INSTITUTE.

Report No. ARF C 222-4
(Progress Report)

DEVELOPMENT OF AN ORALLY EFFECTIVE INSECT REPELLENT

Headquarters
U.S. Army Medical Research and
Development Command
Office of the Surgeon General
Washington 25, D.C.

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Headquarters
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Attention: Major Lee Roy G. Jones

DEVELOPMENT OF AN ORALLY EFFECTIVE INSECT REPELLENT

ARF Project No. C 222
Contract No. DA-49-193-MD-2281

February 1 to April 30, 1963

I. INTRODUCTION

The objective of this program is to develop a mosquito repellent which is effective when given internally, preferably orally, in order to afford better protection than the conventional surface repellents presently available. Development of a bioassay procedure using mice as bait and radioactive albumin as an indicator; the repellent and physical properties of several chemical compounds were previously reported (Report No. ARF C 222-3). The present report describes further progress on this program.

II. METHODS AND RESULTS

A. Determination of Lipid-Water Partition of Potential Repellents

A chemical compound of known weight was added to a separatory funnel containing equal volumes of 0.1 M phosphate buffer (pH 7.4) and purified chloroform. The chloroform was purified by successive washings with 1 N sodium hydroxide, hydrochloric acid, and water. The funnel was vigorously shaken and then allowed to stand for 1 hour for equilibration. A measured portion of the organic phase was placed in a tared watch glass and the solvent was allowed to evaporate for 4 hours at room temperature. The residue was weighed, and the distribution of the compound in the organic phase was determined. The weight of the compound in the water phase was determined by the difference between the initial weight of compound used and the weight in the organic phase. The results are given in Table 1.

B. Synthesis of Potential Insect Repellents

With many natural products, lengthening and branching of the alkyl side chain significantly increases the biological potency of many natural products.[1] In the insect repellent N,N-diethyl-m-toluamide the effects of positional (ortho-,

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[1] Adams, et al. J. Am. Chem. Soc., 71, 1625, 1949.

Table 1

DISTRIBUTION OF POTENTIAL MOSQUITO REPELLENTS
BETWEEN CHLOROFORM AND WATER (BUFFER pH 7.4)

Chemical Compound	g in $CHCl_3$/g in H_2O
Acetamide, N-amyl-alpha-butoxy	5.4
Acetanilide, N propyl	27.8
Acetic acid, chloro-, 2-nitroisobutyl ester	0.2
Acetoacetic acid, ethyl ester, oxime acetate	0.4
Adipamic acid, N,N-diisopropyl-, methyl ester	24.0
Allethrin (chrysanthemumic acid, cis and trans dl, ester with dl-2-allyl-4-hydroxy-3-methyl-3-cyclopenten-1-one	16.7
Anisyl alcohol	7.7
Anthranilic acid, methyl ester	17.5
Benzamide, 2,4-dichloro-N,N-dipropyl	37.5
Benzoic acid, m-amino-, ethyl ester	54.6
Benzyl alcohol, o-chloro-alpha-(trichloro-methyl)	240.0
Benzyl alcohol, o-methoxy	11.7
Benzyl benzoate	500.0
Bicyclo [2.2.1] -5-heptene-2,3-dicarboximide, N-amyl	100.0
Bicyclo [2.2.1] -5-heptene-2,3-dicarboximide, N-(mixed) amyl	150.0
Butyraldehyde, alpha-(2-cyanoethyl)-alpha-ethyl	1.7
Capric acid	∞
Caprylic acid, gamma-ethyl-gamma-formyl	2.1
Citronellic acid	4.0
1,2-Cyclohexanedicarboximide, N-allyl-4-methyl	13.0
Cyclohexanecarboxylic acid, 3-hydroxy-, 2-butoxyethyl ester	1.5
1,2-Cyclonexanedicarboximide, N-sec-butyl	16.0

Table 1 (cont)

Chemical Compounds	g in $CHCl_3$/g in H_2O
1,2-Cyclohexanedicarboximide, N-isobutyl	∞
1,2-Cyclohexanedicarboximide, N-isopropyl-4-methyl	14.6
Cyclohexanemethanol, 1-nitro	6.4
Cyclohexanol, 2-cyclohexyl	36.0
Cyclohexanol, 1-1-ethynylenedi-, diacetate	26.8
Cyclohexanol, 2-phenyl	7.2
4-Cyclohexene-1,2-dicarboximide, N-isobutyl	32.8
4-Cyclohexene-1,2-dicarboximide, N-propyl	45.0
Cyclopentaneacetic acid, 1-hydroxy-, cyclohexyl ester	26.9
Dimethyl phthalate	100.0
m-Dioxane, 5-ethyl-5-nitro-2-(1-propenyl)	0.6
m-Dioxane, 5-ethyl-5-nitro-2-propyl	∞
5-m-Dioxanol, 2-(1-ethyl amyl)	3.1
5-m-Dioxanol, 2-hexyl	3.9
1,4-Dioxaspiro [5.5] hendecan-3-ol, 7-methyl	3.1
Ethanol, 2-[2-(3-methy-2-norcamphanyl-methoxy)ethoxy]	8.4
Famphos	∞
Fencholic acid	7.3
Glutaramic acid, N,N-diethyl-, methyl ester	6.0
Glycine, N-butyl-, isobornyl ester	1.4
Hendecanoic acid	30.9
1,3-Hexanediol, 2-ethyl- (Rutgers 612)	5.0
Hydracrylic acid, beta-pheryl-, isopropyl ester	2.5
Imidan	100.0
Indalone (2H-Pyran-6-carboxylic acid, 3,4-dihydro-2,2-dimethyl-4-oxo-, butyl ester)	∞
Isobutyric acid, alpha-hydroxy-, phenethyl ester	2.4
Methyl Trithion	∞

Table 1 (cont)

Chemical Compound	g in $CHCl_3$/g in H_2O
2-Naphthol, 1, 2, 3, 4-tetrahydro	12.7
2-Naphthol, 1, 2, 3, 4-tetrahydro-1-methyl	35.0
3, 5-Octanediol, 4-ethyl	3.0
Phenethyl alcohol, p-methoxy	15.1
1, 2-Propanediol, 3-isoamoxy	1.6
1, 3-Propanediol, monobenzoate	14.5
Propionanilide, N-butyl	14.0
Ronnel	∞
Ruelene	∞
Senecioic acid, 2- [2-(2-butoxyethoxy)ethoxy]-, ethyl ester	2.1
Succinamic acid, N, N-diethyl-, sec-butyl ester	55.4
Succinamic acid, N, N-diethyl-, propyl ester	100.0
Succinamic acid, N, N-diisopropyl-, isopropyl ester	4.5
Succinamic acid, N, N-diisopropyl-, propyl ester	3.4
Succinamic acid, N, N-dipropyl-, sec-butyl ester	∞
Succinimide, N-amyl	15.0
Thiamine, hydrochloride	0.01
m-Toluamide, N, N-diethyl	200.0
10-Undecenoic acid	60.0
R-2371 "D" (from Stauffer Chem. Co.)	∞

meta-, and para-) isomerism of the methyl group is well known. However, the effect of a longer alkyl chain or the effect of branching on this chain is not well defined. A synthetic study was therefore initiated to determine whether lengthening and branching the alkyl side chain improves the repellent effect. In addition, the diethylamide of 2,6-dimethylbenzoic acid was prepared in order to determine whether crowding of the amide group could retard its effect on metabolism in vivo, and thus retain any repellent potency.

III. EXPERIMENTAL PROCEDURES

A. N,N-Diethyl-2,6-dimethylbenzamide

1. 2,6-Dimethylbenzoic Acid

A 500-ml 2-neck flask was equipped with an adding funnel, "Trubore" stirrer, and a condenser attached to a source of prepurified (P.P.) nitrogen. In the flask was placed 9.5 g (0.39 M) of washed magnesium turnings and about 0.2 g of iodine. The system was flushed with gas while being heated with an open flame to remove oxygen and adsorbed water vapor. After cooling to room temperature, the metal was covered with 30 ml of dry ether and a solution of 60 g (0.34 M) of 2,6-dimethylbromobenzene in 130 ml of dry ether was added at a rate sufficient to cause refluxing. After the spontaneous refluxing ceased, the mixture was refluxed for an additional 3 hours, cooled, and poured onto an excess of powdered dry ice.

After the excess dry ice sublimed, the reaction mixture was treated with 200 ml of 5% sulfuric acid (with cooling). The stirred mixture was heated for 1-2 hours and cooled. The non-aqueous layer was washed with dilute hydrochloric acid, and the dimethylbenzoic acid was extracted with several portions of 10% sodium bicarbonate solution. The bicarbonate solution was acidified with hydrochloric acid, and the precipitated dimethylbenzoic acid was filtered by suction and washed with a small quantity of water.

The solid acid was recrystallized from hot water and dried in a vacuum desiccator. The white crystals of 2,6-dimethylbenzoic acid weighed 28.5 g or 55% of the theoretical amount. It melted at 115-117°C (literature value 116°C).

2. 2,6-Dimethylbenzoyl Chloride

Into a 500-ml single-neck flask equipped with a condenser 44.5 g (0.3 M) 2,6-dimethylbenzoic acid was placed and covered with a 100% excess of purified thionyl chloride. The mixture was heated to refluxing for 7 hours, after which the excess thionyl chloride was removed by distillation under reduced pressure. The acid chloride was collected at 82.9°C/8 mm using a Claisen still head.

3. N,N-Diethyl-2,6-dimethylbenzamide

According to standard methods the 2,6 dimethylbenzoyl chloride was added to a solution of diethylamine in ether. The resulting N,N-diethyl-2,6-dimethylbenzamide was distilled at 90.6°C/0.15 min.

B. N,N-Diethyl-m-(n-propyl)benzamide

1. m-Bromobenzoyl Chloride

In a 2-liter single-neck flask equipped with a condenser 250 g (1.25 M) m-bromobenzoic acid and a 100% excess of purified thionyl chloride was placed. The mixture was heated for about 3 hours after which time the contents were liquid. The solution was refluxed for 8 hours, cooled, and the excess thionyl chloride removed by distillation under reduced pressure. The acid chloride was collected at 110.5°-112°C/8-9mm. The clear liquid weighed 255 g or 93% of the theoretical amount.

2. m-Bromobenzamide

A 3-liter 3-neck flask was equipped with a "Trubore" stirrer, a condenser, a thermometer, and a gas inlet tube (with a plunger for removal of solid at the gas-liquid interface). The flask was charged with 255 g (1.15 M) of m-bromobenzoyl chloride and about 2 liters of dry ether. The solution was stirred and maintained at around 5°C while ammonia (gas) was added. After about 2-1/2 hours the reaction was complete as evidenced by excess ammonia venting through the condenser. The finely divided solid was filtered, washed with water, and recrystallized from methanol-water. The white vacuum-dried crystals melted at 154.5-155°C (literature = 155°C). The amide weighed 215 g or 86% of the theoretical amount.

3. m-Bromo-Propiophenone

A 1-liter 3-neck flask was equipped with a "Trubore" stirrer, and a Dean-Starke trap surmounted by a condenser attached to a source of prepurified nitrogen. The flask was charged with 670 ml of a 3 M solution of ethyl magnesium bromide in ether. The adding funnel was removed and replaced by an erlenmeyer flask attached with wide-bore rubber tubing. From the erlenmeyer flask was added 100 g (0.5 M) of m-bromobenzamide in small portions, at such a rate as to maintain a continuous reflux of the ether. When the addition was complete and the spontaneous refluxing ceased, the ether was heated to refluxing. Dry benzene was added while the distillate was removed continuously through the Dean-Starke trap. When the pot temperature of the mixture rose to 75-80°C, removal of distillate was discontinued and the mixture allowed to reflux for an additional 18 hours.

The cooled reaction mixture was slowly added to a cooled, stirred mixture of 110-ml concentrated sulfuric acid and 500 g of ice, at temperature below 40°C. When addition was complete, the mixture was warmed to 60°C for 1 hour with stirring and the layers separated. The benzene layer was washed with water, dilute bicarbonate, and water till neutral. After drying, the solvent was removed at the water pump with the aid of a rotating evaporator. The residue was distilled through a 6 inch x 1/2 inch Vigreau column in an atmosphere of prepurified nitrogen, aided by an ebullating tube. The m-bromo-propiophenone was collected at 130°-132°C/11 mm.

C. N,N-Diethyl-m-(n-amyl) Benzamide

1. m-Bromobenzoyl Chloride

As described in Section B.

2. m-Bromobenzamide

As described in Section B.

3. m-Bromo-n-valerophenone

The apparatus was set up as described in Section B. n-Butyl magnesium bromide (670 ml of a 3 M solution) in ether was treated with 100 g (0.5 M) of m-bromobenzamide. After addition was complete, dired benzene was added while the distillate was removed. When the pot temperature of the mixture rose to 75°-78°C, refluxing was maintained for 26 hours. The mixture was then allowed to cool to room temperature.

IV. IN VITRO ASSAY PROCEDURE

In order to broaden the scope of assay techniques for insect repellents an investigation into an in vitro method of testing repellents has been undertaken. In this study Aedes aegypti were fed through various membranes, on outdated, citrated whole human blood containing 2 mg/ml of Blancophor dye. The mosquitoes were maintained at room temperature (25°C) during exposure to the bait. The blood temperature was controlled by keeping the surface of the blood above the membrane exposed to circulating air at

various temperatures. The membranes were used in both untreated and in treated state. Treatment consisted of soaking the membranes overnight in a water solution of a wetting agent (Alconox) and washing thoroughly in hot water before use. The blood layer was one-inch deep above the membrane Cages of 50 Aedes aegypti were placed under the membrane for 3 hours. The mosquitoes were then killed by placing them in a box containing dry ice and observed under ultraviolet light. The mosquitoes which had fed through the membrane appeared bright green while those that did not feed remained black. The number of mosquitoes that have fed can thereby be determined. The results of the membranes tested by this procedure are shown in Table 2.

V. CONCLUSIONS AND FUTURE WORK

The Baudruche- and Fourex-treated membranes appear to be the most promising membranes for use in an in vitro assay procedure. The large deviation found in the percent fed using the Fourex membrane is most likely due to the differences in the membrane thickness. If this membrane is not available in uniform thickness, the Baudruche membrane which is more uniform in thickness will be used. During the next quarter we shall standardize the in vitro testing procedure and evaluate compounds in blood-repellent mixtures. Compounds showing repellent activity

Table 2

RESULTS OF FEEDING AEDES AEGYPTI ON OUTDATED, CITRATED WHOLE HUMAN BLOOD THROUGH VARIOUS MEMBRANES

Membrane	Blood Temperature, °C	Number Experiments	% Fed Mean Values
Baudruche	37°	2	4
Baudruche	40°	4	0
Treated Baudruche	37°	7	78 ± 10
Treated Baudruche	34°	6	6
Fourex	37°	2	15
Fourex	40°	4	6
Treated Fourex	34°	5	4
Treated Fourex	37°	14	58 ± 30
Treated Fourex	40°	2	20
Naturalamb	37°	2	0
Naturalamb	40°	2	0
Treated Naturalamb	37°	3	16
Treated Naturalamb	40°	2	3

by this method will be further tested by internal administration into mice. A compound which is active in the in vitro assay and subsequently loses its activity in the in vivo assay would indicate difficulty in adsorption from the site of administration, chemical modification by the body, or lack of excretion through the skin.

The synthesis of potential insect repellents will continue and these compounds will be tested for repellent activity.

Carbon-14-labeled N,N-diethyl-m-toluamide has been obtained. This compound will be administered internally to mice, and its fate in the body determined by physicochemical means. After the metabolic pathways are established, the repellent properties of the metabolites will be determined. The essential structure for an effective internal repellent might thus be established.

VI. PERSONNEL AND RECORDS

The data are recorded in logbooks No. C 13274, and C 13345.

The synthetic work was performed and directed by L. U. Berman assisted by J. Garner.

Respectfully submitted,

ARMOUR RESEARCH FOUNDATION
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